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1: J Cell Biochem 1997 Feb;64(2):258-72 Related Articles, Books, LinkOut



Purification and substrate specificity of polydeoxyribonucleotide kinases isolated from calf thymus and rat liver.

Karimi-Busheri F, Weinfeld M.

Experimental Oncology, Cross Cancer Institute, Edmonton, Alberta, Canada.

Damage to DNA can result in strand breaks with 5'-hydroxyl and 3'-phosphate termini. Before DNA polymerases and ligases can rejoin the broken strands, such termini have to be restored to 5'-phosphate and 3'-hydroxyl groups. Polydeoxynucleotide kinase is an enzyme that may fulfil this function. We have purified the kinases from calf thymus and rat liver to near homogeneity. Based on SDS-polyacrylamide gel electrophoresis and activity gels, the enzymes from both sources are approximately 60-kDa polypeptides. Both enzymes have an acidic pH optimum (5.5-6.0) for kinase activity, and similar pI values (8.5-8.6), and a specificity for DNA. The calf thymus kinase possesses a 3'-phosphatase activity, as has previously been shown for the rat liver enzyme. The minimum size of oligonucleotide that can be labelled is 7-8 nucleotides in length, but the optimal size appears to be > 18 nucleotides. Comparison of phosphorylation of oligo(dA)24 and oligo(dT)24 with oligonucleotides containing a varied nucleotide sequence indicated that the homopolymers are poorer substrates. Unlike the bacteriophage T4 polynucleotide kinase, the mammalian kinases exhibit no preference for 5'-overhanging termini when acting at DNA termini produced by restriction enzymes. With double-stranded oligonucleotide complexes designed to mode single-strand gaps and nicks, the mammalian kinases preferentially phosphorylate the 5'-terminus associated with the gap or nick, in keeping with the idea that the kinases are involved in the repair of DNA single-strand breaks.

PMID: 9027586 [PubMed - indexed for MEDLINE]

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5'phosphorylation of DNA in mammalian cells: identification of a polymin P-precipitable polynucleotide kinase.

Prinos P, Slack C, Lasko DD.

Department of Medicine, McGill University, Montreal, Quebec, Canada.

Proteins that catalyze 5' phosphorylation of an oligodeoxyribonucleotide substrate can be fractionated by polymin P treatment of whole cell extracts of calf thymus glands. Anion exchange chromatography on Q-Sepharose revealed three separable peaks of activity in the polymin P supernatant fraction, and one peak of activity in the Polymin P pellet fraction. The latter activity, Polymin P-precipitable polynucleotide kinase (PP-PNK), was further purified with a 1,500-fold increase of specific activity compared to the crude Polymin P pellet fraction.

Oligonucleotides, a dephosphorylated 2.9-kb EcoRI fragment, and poly(A) were phosphorylated by the enzyme preparation, but thymidine 3' monophosphate was not a substrate. PP-PNK preparations exhibited an apparent KM of 52 microM for ATP and 8 microM for oligo dT25. The enzyme preparation displayed no detectable 3' phosphatase or cyclic 2',3'-phosphohydrolase activities. The sedimentation coefficient of the PP-PNK activity was 3.8S as determined by sucrose density gradient analysis; the Stokes radius was 45 A, leading to an estimated molecular mass of 72 kDa. The enzyme had a pH optimum in the neutral to alkaline range in several buffer systems and is distinct from the DNA kinase with an acidic pH optimum previously described in calf thymus.

PMID: 7642718 [PubMed - indexed for MEDLINE]

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